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# Effects of Parathyroid Hormone and $N^6$,$O^{2'}$-Dibutyryl Cyclic AMP on $Ca^{2+}$ Transport Across the Rabbit Distal Nephron Segments Perfused in vitro

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**Abstract.** Effects on $Ca^{2+}$ transport of parathyroid hormone (PTH) and $N^6$,$O^{2'}$-dibutyryl adenosine 3′,5′-cyclic monophosphate (DB-cAMP) were examined in the rabbit distal nephron segments including the cortical thick ascending limb of Henle's loop (CAL), the connecting tubule (CNT) and the cortical collecting tubule (CCT) by the in vitro perfusion technique. When PTH ($10^{-8}$ mol·$l^{-1}$) was added to the bath, efflux of $Ca^{2+}$ (pmol·$mm^{-1}$·$min^{-1}$) was increased from $6.29 \pm 1.46$ to $7.96 \pm 1.66$ ($P < 0.02$) in the CAL, and from $8.55 \pm 1.30$ to $13.73 \pm 1.24$ ($P < 0.001$) in the CNT, respectively, without changes in influx of $Ca^{2+}$. The effect of PTH on $Ca^{2+}$ transport in the CAL, however, was abolished when phosphate concentration in the medium was reduced from 3.0 to 1.0 mmol·$l^{-1}$. When DB-cAMP ($10^{-3}$ mol·$l^{-1}$) was added to the bath, efflux of $Ca^{2+}$ was also increased from $7.01 \pm 0.83$ to $9.40 \pm 0.82$ ($P < 0.05$) in the CAL, and from $13.11 \pm 0.89$ to $19.74 \pm 0.52$ ($P < 0.005$) in the CNT, respectively. By contrast, neither PTH nor DB-cAMP affected efflux of $Ca^{2+}$ in the CCT. PTH did not affect the transepithelial voltage either in the CAL or in the CNT. But in the CNT, DB-cAMP decreased the voltage from −14.1 to −9.4 mV. The response of adenylate cyclase activity to PTH in the collagenase treated isolated nephron segments was also examined. Significant increases in adenylase cyclase activity were observed in the CAL as well as in the CNT with $10^{-6}$ mol·$l^{-1}$ PTH. These data indicate that PTH stimulates $Ca^{2+}$ transport across the CNT probably via activation of the adenylate cyclase-cyclic AMP system. The hormone may also stimulate $Ca^{2+}$ transport across the CAL in a special condition where plasma phosphate concentration is elevated.

**Key words:** PTH – Cyclic AMP – $C^{2+}$ transport – Adenylate cyclase – Renal diatal tubular segments

## Introduction

It is well known that parathyroid hormone (PTH) decreases urinary excretion of calcium. The stop-flow studies of Widrow and Levinsky [30] suggested that PTH enhances $Ca^{2+}$ transport in the distal nephron. Although the results of micropuncture studies examining the effect of PTH on $Ca^{2+}$ transport across the proximal tubule have been controversial, it is generally accepted that the hormone enhances $Ca^{2+}$ reabsorption in the more distal nephron segments [1–3, 13, 14, 28]. However, exact sites at which PTH enhances $Ca^{2+}$ reabsorption have remained to be established.

Chabardès et al. [9] and Morel et al. [22], who measured adenylate cyclase activity in the isolated single nephron fragments, demonstrated that PTH stimulates adenylate cyclase activity not only in the proximal tubule, but also in the cortical thick ascending limb of Henle's loop (CAL) and in the connecting tubule (CNT)[1]. Based on these findings, they suggested that PTH might modulate $Ca^{2+}$ or phosphate transport across these nephron segments. It has been suggested that the action of PTH is mediated by adenosine 3′,5′-cyclic monophosphate (cAMP). However, the effect of cAMP on $Ca^{2+}$ transport in the kidney has not been firmly established [2, 6, 21].

The present study was designed to investigate the locus and mechanism of action of PTH enhancing $Ca^{2+}$ transport across the distal nephron segments by using the in vitro perfusion technique. Recently, a similar study was reported by Shareghi and Stoner [26]. The results of the present study are in good agreement with their observations in that PTH enhances $Ca^{2+}$ transport across the CNT. But in contrast to their data, I found that the hormone also enhanced $Ca^{2+}$ transport across the CAL in a special condition where ambient phosphate concentration was elevated. In the present study, I also examined the effect of $N^6$,$O^{2'}$-dibutyryl adenosine 3′,5′-cyclic monophosphate (DB-cAMP) on $Ca^{2+}$ transport across the distal nephron segments. In order to correlate the results of the functional study with biochemical changes induced by PTH, adenylate cyclase activity of the single nephron fragments was also measured with the method [29] that is slightly different from the original one [20].

## Methods

### *In vitro Perfusion of Isolated Tubules*

Female white rabbits weighing from 1.5–2.5 kg were used. The animals were fed a regular laboratory chow and had free access to tap water. The method of Burg et al. [7] was used with slight modifications as previously reported [16–18]. The composition of the artificial solution used for either dissection of the renal tubules or perfusion of the tubule was as follows in mmol·$l^{-1}$, NaCl 105, $NaHCO_3$ 25, Na acetate 10, KCl 5.0, $Na_2HPO_4$ 2.25, $NaH_2PO_4$ 0.75, $CaCl_2$ 1.8, $MgCl_2$ 1.0, L-alanine 5.0, and D-glucose 8.3. Rabbit serum was added to the dissection medium in an

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1 The connecting tubule corresponds to the granular segments of the distal convoluted tubule and of the cortical collecting tubule defined by Morel et al. [22]. Validity of this nomenclature has been discussed elsewhere [16]

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amount of 5% (v/v) but not to the bathing fluid while the tubules were being perfused.

The CAL, CNT, and cortical collecting tubule (CCT) were isolated from the renal cortex. The method and criteria to identify the CNT have been already reported [16]. In the present study, the CNT was obtained from the portion of the nephron arcade because of ease of identification.

$^3$H-polyethylene glycol (New England Nuclear) or $^3$H-cyanocobalamin (The Radiochemical Centre, Amersham) was used as a volume marker, and $^{45}$Ca (New England Nuclear) was used to measure the efflux or influx coefficient of $Ca^{2+}$. When $^{45}$Ca was added to perfusate, the efflux coefficient of $Ca^{2+}$, $K_{e(Ca^{2+})}$, was calculated as [12];

$$K_{e(Ca^{2+})} = (V_i/L) \ln (C_i/C_o), \quad (1)$$

where $V_i$ is perfusion rate in ml/s, $L$ is the length of the tubule in cm, and $C_i$ and $C_o$ are concentration of $^{45}$Ca in perfusate and collected fluid, respectively. This equation is valid when there is no net water flux. It has been already shown that in the CNT there was no appreciable water absorption [16]. The efflux of $Ca^{2+}$, $J_{e(Ca^{2+})}$, was calculated as:

$$J_{e(Ca^{2+})} = [Ca^{2+}]_i \, K_{e(Ca^{2+})}, \quad (2)$$

where $[Ca^{2+}]_i$ denotes $Ca^{2+}$ concentration of the perfusate. This calculation is not accurate since changes in $Ca^{2+}$ concentration along the tubular lumen are not taken into consideration. Error introduced by this simplification, however, would be very small when perfusion rate is high and tubular length is short.

When $^{45}$Ca was added to the bath, the influx coefficient, $K_{i(Ca^{2+})}$, and the influx, $J_{i(Ca^{2+})}$, were respectively calculated as:

$$k_{i(Ca^{2+})} = (V_i/L) \ln [C_b/(C_b - C_o)], \quad (3)$$

$$J_{i(Ca^{2+})} = [Ca^{2+}]_b \, K_{i(Ca^{2+})}, \quad (4)$$

where $C_b$ is concentration of $^{45}$Ca in the bath and $[Ca^{2+}]_b$ is concentration of cold $Ca^{2+}$ in the bath.

In order to prevent adsorption of $^{45}$Ca to the glass capillary, the inside of the collecting pipette and the constant volume pipette were coated with silicon using Sylgard 184 with curing agent (Dow Corning). When the sample was transferred into a counting vial, the volumetric pipette was flushed with a solution containing 1% Isotope Cleaner A (Chiyoda, Tokyo, Japan). At the end of each experiment, the perfusion pipette was removed from the perfusion set and placed into the collecting pipette, into which about 300 nl of perfusate was delivered under oil. Calibration of the constant volume pipette used in the experiment was performed by aspirating this solution by the same manner as experimental samples were collected. The ratio of the radioactivity of $^{45}$Ca to that of volume marker was compared to that of bulk solution of the perfusate. By this procedure the recovery rate of $^{45}$Ca from the collecting pipette could be determined by assuming that the recovery of the volume marker was 100%. The radioactivity of the sample was corrected by the recovery rate of $^{45}$Ca. The loss of $^{45}$Ca was mostly within 3%. If the loss of $^{45}$Ca exceeded 5%, the experiment was discarded. In some experiments a small piece of solid EDTA was added to the collected fluid immediately before the constant volume pipette was inserted in order to minimize the loss of $^{45}$Ca.

Since the efflux of $Ca^{2+}$ across the CAL that I have reported previously [17] was higher than the data reported by other laboratories [5, 26], where efflux of $Ca^{2+}$ was measured by appearance of the isotope in the bath, one may argue that $Ca^{2+}$ efflux as calculated from the difference of $^{45}$Ca concentration between perfusate and collected fluid could result in an overestimation of the value because of possible adsorption of the isotope to the collecting pipette. In order to know how much overestimation, if any, could have been made by the method utilizing the equation (1) as compared to the data calculated from the appearance of $^{45}$Ca in the bath, $Ca^{2+}$ efflux across the CAL was determined with these two different methods. The following equation was used when $Ca^{2+}$ efflux was measured from the appearance of $^{45}$Ca in the bath:

$$K'_{e(Ca^{2+})} = (V_i/L) \ln [C_i V_i (C_i V_i - C_b)^{-1}], \quad (5)$$

where $C_b$ denotes the rate of appearance of $^{45}$Ca into the bath in cpm/s. Every 5–15 min the bath was rinsed with 4 ml of warmed fresh solution, and each rinse was collected directly into a scintillation vial, to which was added 10 ml scintillant consisting of 4 g Omnifluor/l of the 2:1 mixture of toluene and Triton X-100). The counting efficiency of this mixture was reduced by 40% as compared to the samples consisting of 1 ml distilled water and 10 ml scintillant. Furthermore, counting efficiency varied when composition of the bathing solution was changed. Therefore, in this particular series of experiments, volume of all samples including standard solution, collected tubular effluent, and pipette calibration were adjusted to 4 ml with the same artificial solution. Results of 7 experiments showed that the efflux coefficient of $Ca^{2+}$ determined by the $^{45}$Ca concentration in the collected fluid ($K_e$) tended to be higher than that determined by the appearance of the isotope in the bath ($K'_e$): $K_e$, 4.15 ± 1.03 vs $K'_e$, 3.61 ± 1.05 ($10^{-7}$ cm$^2 \cdot$ s$^{-1}$). However, this difference was not statistically significant ($K_e - K'_e$, 0.55 ± 0.88).

Transepithelial electrical potential difference (PD) was measured as previously described [18].

The experimental protocol consisted of the control (C) and experimental (E) periods, which were followed by the recovery period (R) in some experiments. About 15–30 min after initiating the perfusion of tubule, three successive collections of samples were performed (C period). Then, either $10^{-8}$ mol$\cdot$l$^{-1}$ (about 8 U$\cdot$l$^{-1}$) synthetic bovine 1–34 PTH (Beckman) or $10^{-3}$ mol$\cdot$l$^{-1}$ DB-cAMP (Sigma) was added to the bath. After a 5–10 min equilibration period, three successive collections were performed (E period). About 5–10 min after removal of the agents from the bath, additional three samples were collected (R period). Constant volume pipettes for sample collection were 100–150 nl in volume, and collection time varied about 5–15 min. Either $K_{e(Ca^{2+})}$ or $K_{i(Ca^{2+})}$ was determined in separate experiments with a similar experimental protocol.

A mean value of three samples represented the value under a given condition of a given tubule. The mean values for individual tubules in each period were then used to calculate mean value ± SEM for the indicated number of tubules. Student's $t$-test for paired samples was used to evaluate the significance of differences between the control and the experimental period.

### *Adenylate Cyclase Activity in Single Nephron Fragments*

In order to confirm the observation of Morel's group [9, 23], adenylate cyclase activity was measured by the method of Torikai and Imai [29] which is slightly different from the original one [20]. Since the detail of the method has been reported [29], the procedure will be briefly summarized. Fragments of rabbit renal tubules were obtained after collagenase treatment as described by Imbert et al. [20] except that renal perfusate consisted of 0.1 g$\cdot$l$^{-1}$ collagenase and 1 g$\cdot$l$^{-1}$ bovine serum albumin in modified bicarbonate Krebs-Ringer solution. Each tubule was transferred to an incubation well (Microtest, Falcon). After elimination of dissection medium, an aliquot of 0.5 µl hypotonic medium was added. The tubule was made permeable by twice freezing and thawing. Incubation was performed at 30°C for 30 min after addition of 2 µl of incubation medium which consisted of ATP 0.5 mmol$\cdot$l$^{-1}$, creatine phosphate 20 mmol$\cdot$l$^{-1}$, creatine kinase 1 g$\cdot$l$^{-1}$, theophylline 10 mmol$\cdot$l$^{-1}$, EDTA 0.25 mmol$\cdot$l$^{-1}$, $MgCl_2$ 3.8 mmol$\cdot$l$^{-1}$, and Tris HCl buffer (pH 7.4) 100 mmol$\cdot$l$^{-1}$. The reaction was stopped by quickly freezing and adding 5 µl of 10 mmol$\cdot$l$^{-1}$ EDTA. The generated cAMP was measured by a radioimmunoassay after succinylations [15]. Adenylate cyclase activity was expressed as fmol of cAMP generated during 30 min incubation period per mm of the tubular length.

The values were expressed as mean ± SEM. Student's $t$-test for non-paired samples was used to evaluate the significance of differences.

## Results

### *Effect of PTH on $Ca^{2+}$ Transport*

Effect of $10^{-8}$ mol$\cdot$l$^{-1}$ PTH (about 8 U$\cdot$l$^{-1}$) added to the bath on $Ca^{2+}$ efflux was examined in the CAL, CNT and CCT. The results are summarized in Table 1 and Fig. 1. A small but significant increase in $Ca^{2+}$ efflux was observed in the CAL after PTH was added to the bath. A marked increase in $Ca^{2+}$

**Table 1.** Effect of PTH ($10^{-8}$ mol·l$^{-1}$ in the bath) on $Ca^{2+}$ efflux across the CAL, CNT, and CCT

| Segment | $N$ | Length mm | | $V_i$ nl·min$^{-1}$ | $K_{e(Ca^{2+})}$ $10^{-7}$·cm$^2$·s$^{-1}$ | $J_{e(Ca^{2+})}$ pmol·mm$^{-1}$·min$^{-1}$ |
|---|---|---|---|---|---|---|
| CAL | (7) | 1.25 ± 1.11 | C | 14.57 ± 1.81 | 5.82 ± 1.35 | 6.29 ± 1.46 |
| | | | E | 14.17 ± 1.89 | 7.37 ± 1.54 | 7.96 ± 1.66 |
| | | | $\Delta$ | | 1.56 ± 0.43 | 1.68 ± 0.46 |
| | | | $P$ | | < 0.02 | < 0.02 |
| CNT | (9) | 0.47 ± 0.03 | C | 10.99 ± 1.54 | 7.92 ± 1.20 | 8.55 ± 1.30 |
| | | | E | 11.64 ± 1.37 | 12.71 ± 1.15 | 13.73 ± 1.24 |
| | | | $\Delta$ | | 4.79 ± 0.93 | 5.17 ± 1.00 |
| | | | $P$ | | < 0.001 | < 0.001 |
| CCT | (6) | 1.73 ± 0.18 | C | 14.64 ± 1.62 | 3.98 ± 0.34 | 4.30 ± 0.37 |
| | | | E | 13.26 ± 0.90 | 3.89 ± 0.44 | 4.20 ± 0.48 |
| | | | $\Delta$ | | − 0.09 ± 0.16 | − 0.10 ± 0.17 |
| | | | $P$ | | NS | NS |

CAL = cortical thick ascending limb of Henle, CNT = connecting tubule, CCT = cortical collecting tubule, $V_i$ = perfusion rate, $K_{e(Ca^{2+})}$ = efflux coefficient of calcium, $J_{e(Ca^{2+})}$ = efflux of calcium, C = control period, E = experimental ($10^{-8}$ mol·l$^{-1}$ PTH), $\Delta$ = E − C, $P$ = probability of the difference, NS = not significant, $N$ = number of tubules

**Table 2.** Effect of PTH ($10^{-8}$ mol·l$^{-1}$ in the bath) on $Ca^{2+}$ influx across the CAL and CNT

| Segment | $N$ | Length mm | | $V_i$ nl·min$^{-1}$ | $K_{i(Ca^{2+})}$ $10^{-7}$·cm$^2$·s$^{-1}$ | $J_{i(Ca^{2+})}$ pmol·mm$^{-1}$·min$^{-1}$ |
|---|---|---|---|---|---|---|
| CAL | (5) | 1.58 ± 0.02 | C | 16.48 ± 1.15 | 1.62 ± 0.29 | 1.75 ± 0.31 |
| | | | E | 15.66 ± 0.70 | 1.61 ± 0.20 | 1.74 ± 0.22 |
| | | | $\Delta$ | | −0.01 ± 0.35 | −0.01 ± 0.38 |
| | | | $P$ | | NS | NS |
| CNT | (5) | 0.58 ± 0.07 | C | 14.05 ± 0.29 | 5.71 ± 1.28 | 6.17 ± 1.38 |
| | | | E | 13.42 ± 0.55 | 5.07 ± 1.07 | 5.48 ± 1.16 |
| | | | $\Delta$ | | −0.64 ± 0.39 | −0.69 ± 0.42 |
| | | | $P$ | | NS | NS |

$K_{e(Ca^{2+})}$ = influx coefficient of calcium, $J_{i(Ca^{2+})}$ = influx of calcium. Other abbreviations and symbols are same as in Table 1

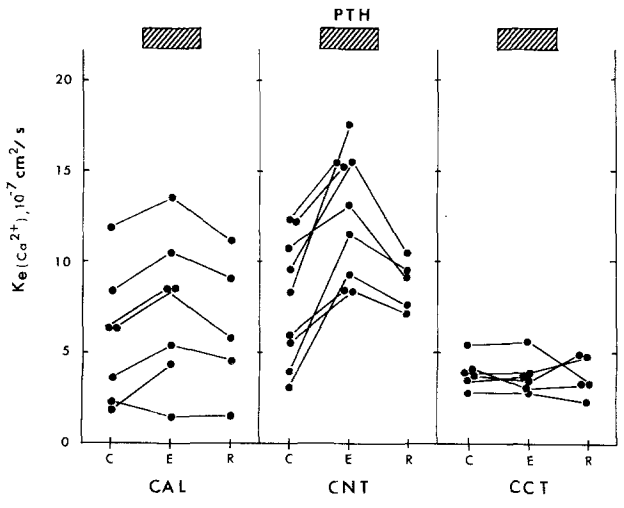


**Fig. 1.** Effect of $10^{-8}$ mol·l$^{-1}$ PTH added to the bath on efflux coefficient of $Ca^{2+}$ ($K_e$) in the CAL, CNT, and CCT. *C* control, *E* experimental, *R* recovery period

efflux with PTH was observed in the CNT. These responses were reversible as depicted in Fig. 1, where individual values of $K_e$ are shown. On the other hand, PTH did not show any effect on $Ca^{2+}$ efflux in the CCT.

Shareghi and Stoner [26] recently reported that PTH decreased rather than increased $Ca^{2+}$ transport across the CAL. Since in their study, phosphate concentration in the bath was much lower than was in the present study, effect of PTH on $Ca^{2+}$ efflux across the CAL was examined when the concentration of phosphate of artificial solution was reduced from 3.0 to 1.0 mmol·l$^{-1}$. Under this circumstance, $K_e$ ($10^{-7}$ cm$^2$·s$^{-1}$) was unchanged with addition of $10^{-8}$ mol·l$^{-1}$ PTH (5.34 ± 0.29 in control vs 5.24 ± 1.17 after PTH, $n$ = 5).

Effect of PTH in the bath on $Ca^{2+}$ influx was examined in the CAL and the CNT in a separate series of experiments. As summarized in Table 2, PTH did not influence $Ca^{2+}$ influx in both segments. It should be noted that the influx coefficient of $Ca^{2+}$ in the CAL was low and about the same as the value previously reported [17]. On the other hand, in the CNT the influx coefficient was relatively large, suggesting that this segment may be highly permeable to $Ca^{2+}$.

### *Effect of DB-cAMP on $Ca^{2+}$ Efflux*

The efflux of $^{45}Ca$ was measured in the CAL, CNT and CCT before and after addition of $10^{-3}$ mol·l$^{-1}$ DB-cAMP to the

**Table 3.** Effects of dibutyryl cAMP ($10^{-3}$ mol·l$^{-1}$ in the bath) on $Ca^{2+}$ efflux across the CAL, CNT and CCT[a]

| Segment | $N$ | Length mm | | $V_i$ nl·mm$^{-1}$ | $K_{e(Ca^{2+})}$ $10^{-7}$·cm$^2$·s$^{-1}$ | $J_{e(Ca^{2+})}$ pmol·mm$^{-1}$·min$^{-1}$ |
|---|---|---|---|---|---|---|
| CAL | (6) | 1.83 ± 0.16 | C | 10.44 ± 1.80 | 6.49 ± 0.77 | 7.01 ± 0.83 |
| | | | E | 10.80 ± 1.43 | 8.70 ± 0.76 | 9.40 ± 0.82 |
| | | | $\Delta$ | | 2.21 ± 0.71 | 2.39 ± 0.77 |
| | | | $P$ | | < 0.05 | < 0.05 |
| CNT | (6) | 0.58 ± 0.03 | C | 14.28 ± 1.37 | 12.14 ± 0.82 | 13.11 ± 0.89 |
| | | | E | 14.40 ± 1.94 | 18.20 ± 0.48 | 19.74 ± 0.52 |
| | | | $\Delta$ | | 6.06 ± 0.98 | 6.54 ± 1.06 |
| | | | $P$ | | < 0.005 | < 0.005 |
| CCT | (5) | 1.68 ± 0.22 | C | 13.06 ± 0.47 | 5.91 ± 0.88 | 6.38 ± 0.95 |
| | | | E | 10.41 ± 1.45 | 5.64 ± 0.81 | 6.09 ± 0.87 |
| | | | $\Delta$ | | − 0.27 ± 0.26 | − 0.29 ± 0.28 |
| | | | $P$ | | NS | NS |

[a] Abbreviations and symbols are same as in Table 1

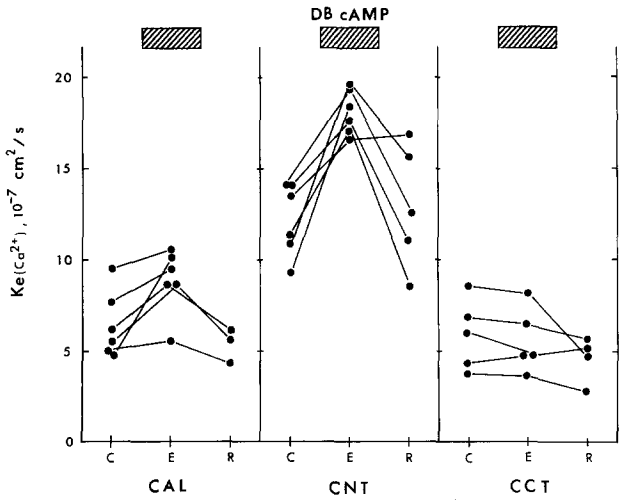


**Fig. 2.** Effect of $10^{-3}$ mol·l$^{-1}$ DB-cAMP added to the bath on efflux coefficient of $Ca^{2+}$ ($K_e$) in the CAL, CNT, and CCT. *C* control, *E* experimental, *R* recovery period

**Table 4.** Effects of PTH ($10^{-8}$ mol·l$^{-1}$ in the bath) and DB-cAMP ($10^{-3}$ mol·l$^{-1}$ in the bath) on transepithelial electrical potential difference (PD) of the CAL and the CNT

| Segment | | PD (mV) |
|---|---|---|
| CAL | Control | + 5.1 ± 0.74 (5) |
| | PTH | + 5.0 ± 0.73 (5) |
| | Control | + 4.8 ± 0.57 (5) |
| | DB-cAMP | + 4.9 ± 0.63 (5) |
| CNT | Control | −14.2 ± 1.55 (5) |
| | PTH | −14.3 ± 1.44 (5) |
| | Control | −14.1 ± 1.37 (5) |
| | DB-cAMP | − 9.4 ± 0.81*(5) |

* $P < 0.001$ as compared to control

bath. The results are summarized in Table 3 and in Fig. 2. A moderate increase in $Ca^{2+}$ efflux was observed in the CAL after DB-cAMP was added to the bath. A marked increase in $Ca^{2+}$ efflux was noted in the CNT with DB-cAMP. In contrast, DB-cAMP did not affect $Ca^{2+}$ efflux across the CCT. Thus, the stimulation with DB-cAMP was very similar to that with PTH.

In order to ensure the specificity of the effect of DB-cAMP, an effect of another nucleotide, dibutyryl guanosine 3′,5′-cyclic monophosphate (DB-cGMP) was also examined in the CNT. The $Ca^{2+}$ efflux (pmol·mm$^{-1}$·min$^{-1}$) was unchanged by $10^{-3}$ mol·l$^{-1}$ DB-cGMP (7.86 ± 0.52 in control vs 8.03 ± 0.57 after DB-cGMP, $n = 5$).

### *Effects of PTH and DB-cAMP on PD*

In a separate series of experiments, effects of $10^{-8}$ mol·l$^{-1}$ PTH and $10^{-3}$ mol·l$^{-1}$ DB-cAMP on the PD of the CAL and CNT were examined. In most experiments, effects of PTH and DB-cAMP were observed successively in the same tubule. The results are summarized in Table 4. PTH did not cause any significant changes in PD in all segments, whereas DB-cAMP caused a decrease in PD in the CNT. Since it has been reported that both isoproterenol and vasopressin decreased PD of the CNT and the CCT [18], the effect of DB-cAMP on the PD of these segments may correspond to the effect of such hormones rather than that of PTH.

### *Effect of PTH on Nephron Adenylate Cyclase Activity*

The studies of Chabardès et al. [9] and Morel et al. [22] were re-examined with a somewhat different technique. Adenylate cyclase activity was determined with or without PTH in various nephron segments including the medullary thick ascending limb (MAL), CAL, distal convoluted tubule (DCT), CNT, and CCT. Nonspecific response to NaF was also examined. The results are summarized in Table 5. A marked increase in adenylate cyclase activity was observed in the CNT in the presence of $10^{-6}$ mol·l$^{-1}$ (about 800 U·l$^{-1}$) PTH. A small but significant increase in the activity was observed in the CAL. In contrast, adenylate cyclase activity was not stimulated with PTH in the MAL, DCT, and CCT. These findings are qualitatively in good agreement with those of Chabardès et al. [9] and Morel et al. [22].

**Table 5.** Effect of PTH ($10^{-6}$ mol·$l^{-1}$) on adenylate cyclase activity of rabbit nephron segments

| Segment | cAMP (fmol·$mm^{-1}$ per 30 min) | | |
|---|---|---|---|
| | Control | PTH ($10^{-6}$ mol·$l^{-1}$) | NaF ($10^{-2}$ mol·$l^{-1}$) |
| PCT* | 42.8 ± 6.7 (15) | 285.0 ± 44.0** (10) | 310.0 ± 67.0 (20) |
| MAL | 59.5 ± 7.6 (8) | 50.0 ± 5.5 (10) | 687.9, 434.5 (2) |
| CAL | 46.1 ± 9.1 (8) | 134.4 ± 17.3** (8) | 167.6, 222.1 (2) |
| DCT | 130.2 ± 10.9 (10) | 135.9 ± 13.8 (10) | 1890 ± 519 (3) |
| CNT | 81.9 ± 10.9 (10) | 809.1 ± 136.0** (10) | 806.5 ± 91.0 (3) |
| CCT | 51.1 ± 9.7 (8) | 56.2 ± 10.2 (8) | 721.9, 680.5 (2) |

PCT = proximal convoluted tubule, MAL = medullary thick ascending limb, DCT = distal convoluted tubule, corresponding to the bright portion defined by Morel et al. [23]. Other abbreviations and symbols are same as in Table 1.
* Data by Torikai and Imai [26] are cited for comparison. ** $P < 0.001$ as compared to Control

## Discussion

Morel and his colleagues [9, 22] have demonstrated that PTH stimulates adenylate cyclase activity not only in the proximal tubule, but also in the CAL and the CNT of the rabbit kidney. Although they initially reported that PTH stimulates adenylate cyclase activity also in the DCT [9], a later study revealed that the response was confined to the granular portion of the DCT [22], the CNT. The present study confirms their observation by using a slightly different method.

Stop-flow studies in the dog have suggested that PTH enhances $Ca^{2+}$ absorption in the distal portion of the nephron [30]. Micropuncture or in vivo microperfusion studies examining the effect of thyroparathyroidectomy (TPTX) or PTH on $Ca^{2+}$ transport are in accord with this notion [1–3, 13, 28]. In the TPTX rat, urinary fractional excretion of $Ca^{2+}$ was higher than in intact rats despite the fact that the fraction of the filtered load of $Ca^{2+}$ remaining in the late distal tubule was the same [1]. Sutton et al. [28] found in intact as well as in TPTX dogs that urinary fractional excretion of $Ca^{2+}$ was reduced with PTH in spite of consistent increases in amount of $Ca^{2+}$ delivered to the distal puncture site. These findings suggest that PTH may enhance $Ca^{2+}$ reabsorption in the terminal nephron segments beyond the random or late distal puncture site. Thus the collecting tubule and the other distal segments inaccessible for micropuncture may be the candidates for the site of action of PTH.

The results of the present study indicate that the CNT is the major site at which PTH enhances $Ca^{2+}$ absorption. Administration of PTH in the bath markedly increased efflux of $Ca^{2+}$ without influencing influx in the CNT. By contrast, the hormone did not affect $Ca^{2+}$ efflux across the CCT. These observations are qualitatively in good agreement with those recently reported by Shareghi and Stoner [26]. They found that PTH at the concentration of 30 U·$l^{-1}$ stimulated net $Ca^{2+}$ absorption in the CNT (granular portion of the cortical collecting tubule, CCTg, according to their nomenclature) but not in the CCT. According to their data an increase in net $Ca^{2+}$ flux with PTH was about 0.70 pmol·$mm^{-1}$·$min^{-1}$ (a mean of three experiments). Although I have not directly measured net $Ca^{2+}$ flux, a change in $Ca^{2+}$ efflux may correspond to an increase in net $Ca^{2+}$ flux, since $Ca^{2+}$ influx was unchanged with PTH. The data in Table 1 show that an increase of $Ca^{2+}$ efflux was 5.17 pmol·$mm^{-1}$·$min^{-1}$, a value that is much higher than theirs. In addition, the control value of $Ca^{2+}$ efflux was much higher in the present study. Since in this study $Ca^{2+}$ efflux was calculated by the difference of $^{45}Ca$ concentration between perfusate and collected fluid, $Ca^{2+}$ efflux might be overestimated if $^{45}Ca$ is adsorbed to the glass wall of the collecting pipette. I have tried to eliminate such an error by siliconizing the wall of glass pipettes. Even with this caution, it sometimes happened that a small but significant amount (usually 1–2%) of $^{45}Ca$ was adsorbed to the glass wall of the collecting pipette. Therefore, at the end of each experiment, perfusate was delivered from the perfusion pipette into the collecting pipette, and the recovery rate of $^{45}Ca$ was always checked by comparing the recovery rate of a volume marker. By using this recovery rate of $^{45}Ca$ adsorbed to the glass can be corrected.

The results of the study in which two different methods to measure $Ca^{2+}$ efflux were compared showed that the values calculated from the disappearance of $^{45}Ca$ tended to be slightly higher than those calculated from the appearance of $^{45}Ca$ in the bath. But this difference was too small to explain the higher $Ca^{2+}$ efflux obtained in my laboratory [17]. $Ca^{2+}$ transport across the proximal straight tubule [24] and the CAL of rabbits [17] and the distal convoluted tubule of rats [11] has been shown to be dependent on amount of $Ca^{2+}$ delivered to the segments. Therefore, higher perfusion rate used in this study may, at least in part, account for higher value of $Ca^{2+}$ efflux. In addition to the differences in perfusion rates, some unidentified technical differences might also contribute to the discrepancies. In spite of quantitative difference of the data, it is evident that PTH increases $Ca^{2+}$ absorption across the CNT. Although Shareghi and Stoner [26] reported that PTH also stimulated net $Ca^{2+}$ flux across the DCT, the perfused segment was heterogenous containing the granular portion, probably the CNT. In accord with the observation of Morel et al. [22], I found that PTH did not stimulate adenylate cyclase activity in the DCT. This would suggest that the DCT is not a target of PTH. I have tried to perfuse the segments of the DCT with bright appearance, but they were too short to obtain reliable flux data. Therefore, at present time it is impossible to determine whether PTH affects $Ca^{2+}$ transport only in the granular portion.

The results of micropuncture studies examining the effect of PTH on $Ca^{2+}$ transport in the loop of Henle are controversial. Buerkert et al. [3] observed in TPTX rat that fractional $Ca^{2+}$ reabsorption at the distal tubule was not significantly different from that in intact rats in spite of a significant reduction in the proximal absorption. Thus, $Ca^{2+}$ reabsorption in the loop of Henle was apparently enhanced in TPHX rats, suggesting that PTH does not stimulate $Ca^{2+}$

reabsorption in the loop of Henle. The observation of Sutton et al. [28] in the dog was somewhat different. They found that administration of PTH to intact as well as TPTX dogs enhanced fractional reabsorption of $Ca^{2+}$ measured at the puncture site in the distal tubule, while $Ca^{2+}$ reabsorption in the proximal tubule was unchanged. These data suggest that PTH enhances $Ca^{2+}$ reabsorption somewhere in the loop of Henle provided that the hormone has no effect on the early DCT.

The results of the in vitro microperfusion studies examining the effect of PTH on $Ca^{2+}$ transport across the thick ascending limb of Henle's loop are perplexing. Shareghi and Stoner [26] reported that $10-20\,U\cdot l^{-1}$ of PTH rather decreased $Ca^{2+}$ transport across the CAL. On the other hand, Bourdeau and Burg [4] showed that PTH increased $Ca^{2+}$ efflux across the CAL without influencing the PD. More recently, Shareghi and Agus [25] reported that a small increase in net $Ca^{2+}$ flux was observed when $1\,kU\cdot l^{-1}$ of PTH was added to the bath. Suki and Rouse [27] also reported that $0.1\,kU\cdot l^{-1}$ of PTH was effective in enhancing $Ca^{2+}$ transport across the CAL, but not across the medullary thick ascending limb. In the present study, I found that PTH enhanced $Ca^{2+}$ transport in the CAL only when the ambient phosphate concentration was high. However, owing to a large scatter of the data, it is difficult to conclude with certainty about the role of phosphate in the modulation of anticalciuric action of PTH.

Although Shareghi and Stoner [26] reported that PTH decreased net $Ca^{2+}$ transport in association with a decrease in PD, I could not find any effect of PTH on PD of the CAL. PTH also did not influence PD of the CNT. Although the PD was not measured in parallel with flux measurement, it seems that the effect of PTH in enhancing $Ca^{2+}$ transport is not associated with changes in PD, supporting our view that $Ca^{2+}$ transport across the CAL is, at least in part, active process.

A large body of evidence has supported the hypothesis that the phosphaturic effect of PTH is mediated by cAMP via stimulation of adenylate cyclase activity. However, evidence that cAMP mediates PTH action on $Ca^{2+}$ transport has not been entirely convincing. Kuntziger et al. [21] failed to demonstrate a significant decrease in $Ca^{2+}$ excretion in the PTX rat with cAMP at a dose sufficient to increase phosphate excretion. Although Agus et al. [2] also could not observe a decrease in urinary $Ca^{2+}$ excretion in the intact dog with DB-cAMP, they found a significant decrease in $Ca^{2+}$ reabsorption with this agent in the proximal tubule. These observations, therefore, suggested that DB-cAMP enhanced $Ca^{2+}$ reabsorption in the distal nephron segments. More recently, Burnatowska et al. [6] demonstrated in the hamster that administration of either cAMP or DB-cAMP significantly reduced fractional $Ca^{2+}$ excretion. The present study provides direct evidence that DB-cAMP mimics the action of PTH enhancing $Ca^{2+}$ absorption in the CAL as well as in the CNT. Since it is unknown how much cAMP can penetrate into the cells, a large amount of DB-cAMP was added to the bath in this study. Therefore, it is uncertain whether physiological concentration of cAMP can in fact mimic the action of PTH.

The effects of DB-cAMP on PD of the distal nephron segments were dissociated from those of PTH (Table 4). This would not necessarily mean that DB-cAMP does not mimic the action of PTH in the CNT. Rather, it would mean that the PD lowering effect of DB-cAMP in the CNT might reflect the response of this segments to agents other than PTH such as vasopressin or catecholamines.

In conclusion, PTH increases tubular absorption of $Ca^{2+}$ by selectively increasing efflux of $Ca^{2+}$ without influencing influx mainly in the CNT. PTH may also increase $Ca^{2+}$ absorption across the CAL to a small extent. This effect of PTH may be dependent on ambient phosphate concentration. Stimulation of $Ca^{2+}$ transport by PTH may be, at least in part, mediated by an activation of adenylate cyclase. The mechanism whereby cAMP modulates $Ca^{2+}$ transport across the renal tubule, however, remains to be determined. Although it is clear that in the rabbit kidney physiological responses to PTH were in parallel with adenylate cyclase responses, it is too early to generalize the present results to all mammals. Distribution of PTH sensitive adenylate cyclase has been shown to be different among species [8, 10, 23]. For instance, PTH sensitive adenylate cyclase was demonstrable in the bright portion of the DCT of the mouse, rat and man, but not of the rabbit. Further studies are necessary to examine whether there are species differences in the tubular $Ca^{2+}$ transport in response to PTH.

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## References

1. Agus ZS, Chiu PJS, Goldberg M (1977) Regulation of urinary calcium excretion in the rat. Am J Physiol 232:F545–F549
2. Agus ZS, Gardner LB, Beck LH, Goldberg M (1973) Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium, and phosphate. Am J Physiol 224:1143–1148
3. Buerkert J, Marcus D, Jamison RL (1972) Renal tubular calcium reabsorption after parathyroidectomy. J Clin Invest 51:17a
4. Bourdeau JE, Burg MB (1980) Effect of PTH on calcium transport across the cortical thick ascending limb of Henle's loop. Am J Physiol 239:F121–F126
5. Bourdeau JE, Burg MB (1979) Voltage dependence of calcium transport in the thick ascending limb of Henle's loop. Am J Physiol 236:F357–F364
6. Burnatowska MA, Harris CA, Sutton RAL, Dirks JH (1977) Effects of PTH and cAMP on renal handling of calcium, magnesium, and phosphate in the hamster. Am J Physiol 233:F514–F518
7. Burg MB, Grantham J, Abramow M, Orloff J (1966) Preparation and study of fragments of single rabbit nephrons. Am J Physiol 210:1293–1298
8. Chabardès D, Gagnan-Brunette M, Imbert-Teboul M, Gontcharevskaia O, Montegut M, Clique A, Morel F (1980) Adenylate cyclase responsiveness to hormones in various portions of the human nephron. J Clin Invest 65:439–448
9. Chabardès D, Imbert M, Motégut M, Morel F (1975) PTH sensitive adenyl cyclase activity in different segments of the rabbit nephron. Pflügers Arch 354:229–239
10. Chabardès D, Imbert-Tenoul M, Gagnan-Brunette M, Morel F (1978) Different hormonal target sites along the mouse and rabbit nephrons. In: Guder WG, Schmidt U (eds) Current problems in clinical biochemistry, Hans Huber, Berlin, Stuttgart, Wien, pp 447–454
11. Costanzo LS, Windhager EE (1978) Calcium and sodium transport by the distal convoluted tubule of the rat. Am J Physiol 235:F492–F506
12. Grantham JJ, Burg MB (1966) Effect of vasopressin and cyclic AMP on permeability of isolate collecting tubules. Am J Physiol 221:255–259
13. Greger R, Lang F, Oberleithner H (1978) Distal site of calcium reabsorption in the rat nephron. Pflügers Arch 374:153–157

14. Harris CA, Burnatowska MA, Seely JF, Sutton RAL, Quamme GA, Dirks JH (1979) Effects of parathyroid hormone on electrolyte transport in the hamster nephron. Am J Physiol 236: F342–F348
15. Honma M, Satoh T, Takezawa J, Ui M (1977) An ultrasensitive method for the simultaneous determination of cyclic AMP and cyclic GMP in small-volume samples from blood and tissue. Biochem Med 18:257–273
16. Imai M (1977) Function of the thin ascending limb of Henle of rats and hamsters perfused in vitro. Am J Physiol 232: F201–F209
17. Imai M (1978) Calcium transport across the thick ascending limb of Henle's loop perfused in vitro. Pflügers Arch 374:255–263
18. Imai M (1979) The connecting tubule: a functional subdivision of the rabbit distal nephron segments. Kidney Int 15:346–356
19. Imai M, Kokko J (1974) Sodium chloride, urea and water transport in the thin ascending limb of Henle. Generation of osmotic gradients by passive diffusion of solutes. J Clin Invest 53:393–402
20. Imbert M, Chabardès D, Motégut M, Clique A, Morel F (1975) Adenylate cyclase activity along the rabbit nephron as measured in single isolated segments. Pflügers Arch 354:213–228
21. Kuntziger H, Amiel C, Roinel N, Morel F (1974) Effects of parathyroidectomy and cyclic AMP on renal transport of phosphate, calcium, and magnesium. Am J Physiol 227:905–911
22. Morel F, Chabardès D, Imbert M (1976) Functional segmentation of the distal tubule by microdetermination of hormone dependent adenylate cyclase activity. Kidney Int 9:264–277
23. Morel F, Chabardès D, Imbert-Teboul M (1978) Heterogeneity of hormonal control in the distal nephron. In Proc 7th Int Congr Nephrol (Montreal), S Karger, Basel, pp 209–216
24. Rouse D, Ng RCK, Suki WN (1980) Calcium transport in the pars recta and thin descending limb of Henle of the rabbit, perfused in vitro. J Clin Invest 65:37–42
25. Shareghi GR, Agus ZS (1979) PTH sensitive magnesium transport in the rabbit cortical thick ascending limb of Henle (CTAL). Kidney Int 16:837
26. Shareghi GR, Stoner LC (1978) Calcium transport across segments of the rabbit distal nephron in vitro. Am J Physiol 235: F367–F375
27. Suki WN, Rouse D (1979) Heterogeneity of calcium transport in the medullary and coreical segments of the thick ascending limb of Henle. Kidney Int 16:840
28. Sutton RAL, Wong NLM, Dirks JH (1976) Effect of parathyroid hormone on sodium and calcium transport in the dog nephron. Clin Sci Mol Med 51:345–351
29. Torikai S, Imai M (1979) A simple method to determine adenylate cyclase activity in isolated single nephron segments by radioimmunoassay for succinyl adenosine 3′,5′-cyclic monophosphate. Tohoku J Exp Med 129:91–99
30. Widrow SH, Levinsky NG (1962) The effect of parathyroid extract on renal tubular calcium reabsorption in the dog. J Clin Invest 41:2151–2159

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